



# Active penetration of *Trypanosoma cruzi* into host cells: historical considerations and current concepts

Wanderley de Souza<sup>1,2</sup> and Tecia M. Ulisses de Carvalho<sup>1\*</sup>

<sup>1</sup> Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

<sup>2</sup> Instituto Nacional de Metrologia, Qualidade e Tecnologia, Rio de Janeiro, Brazil

## Edited by:

Ricardo T. Gazzinelli, University of Massachusetts Medical School, USA

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## \*Correspondence:

Tecia M. Ulisses de Carvalho,  
Laboratório de Ultraestrutura Celular  
Hertha Meyer, Instituto de Biofísica  
Carlos Chagas Filho, Universidade  
Federal do Rio de Janeiro, CCS-Bloco  
G, Ilha do Fundão, 21941-900  
Rio de Janeiro, Brazil.  
e-mail: tecia@biof.ufrj.br

In the present short review, we analyze past experiments that addressed the interactions of intracellular pathogenic protozoa (*Trypanosoma cruzi*, *Toxoplasma gondii*, and *Plasmodium*) with host cells and the initial use of the term active penetration to indicate that a protozoan “crossed the host cell membrane, penetrating into the cytoplasm.” However, the subsequent use of transmission electron microscopy showed that, for all of the protozoans and cell types examined, endocytosis, classically defined as involving the formation of a membrane-bound vacuole, took place during the interaction process. As a consequence, the recently penetrated parasites are always within a vacuole, designated the parasitophorous vacuole (PV).

**Keywords:** *Trypanosoma cruzi*, endocytosis, active penetration, phagocytosis, Chagas disease

## INTRODUCTION AND EARLY STUDIES

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zoan in tissue culture were developed. The first approach was described by Kofoed et al. (1935), who showed that the protozoan could survive and multiply in cultures of heart cells from mouse and rat embryos. A subsequent study by Romãña and Meyer (1942) using chick embryo heart cell cultures described the behavior of *T. cruzi* in tissue cultures in detail. Their *in vitro* observations of the interaction process in both living and fixed cultures led the authors to describe two mechanisms of cell infection by the protozoan: active penetration and phagocytosis. In this classic paper, it was stated that “in general, the active penetration was more visible with metacyclic forms that, with their great motility, easily crossed the cell surface, penetrating into the protoplasm of fibroblasts and myocytes.” To the best of our knowledge, this is the first reference to the active penetration of parasitic protozoans into cells. Six years later, Meyer and Xavier de Oliveira (1948) published a paper and a small book confirming the initial observations and reported that the parasite “can touch the surface of the host cells without penetration. Occasionally, they adhere to the cell surface and suddenly penetrate into the cell.” These observations were more clearly demonstrated in a classic film by H. Meyer and A. Barasa. Part of that film which is kept in our laboratory (founded in 1940 by Hertha Meyer) is available at the site of the Brazilian Society of Protozoology ([www.sbpz.org.br](http://www.sbpz.org.br)). The same basic idea initially proposed by Meyer and colleagues was presented in 1973 by Dvorak and Hyde (Dvorak and Hyde, 1973; Hyde and Dvorak, 1973) based on observations of the interaction of *T. cruzi* trypomastigotes of the Ernestina strain with secondary bovine embryo

skeletal muscle cells (BESM) and HeLa cells obtained through

in which mechanical activity of the protozoan is prevalent, and the parasites penetrate into the cell through the plasma membrane. According to these studies, the parasites enter cells posterior end first.

It is important to note, although it is not the focus of the present review, that the same basic idea of active penetration has also been applied to other protozoans, such as *Toxoplasma gondii* and *Plasmodium*, which were again analyzed first by Nery Guimarães and Meyer (1942) and Dvorak et al. (1975).

## SECOND PHASE

One of the authors of this review (WS) joined the Hertha Meyer’s laboratory in 1969 and began the first studies addressing the interaction of *T. cruzi* with chick embryo heart muscle cells and macrophages using electron microscopy. Jim Dvorak visited the laboratory several times between 1972 and 1980, and intense discussions about the concept of active penetration took place at that time. With enthusiasm, Jim defended the idea that *T. cruzi* and *Plasmodium* are able to generate a transient tunnel-like structure in the host cell plasma membrane that is sealed immediately after parasite internalization, and the parasite then establishes intimate contact with the host cell cytoplasm. Up to this point, electron microscopy had not been used to analyze the parasitic protozoan–host cell interaction process. However, a few images obtained in our laboratory showed that recently penetrated trypomastigotes were not in contact with the myofibers of heart muscle cells but were instead located within a membrane-bound vacuole. These observations were made approximately in 1972

when Nadia Nogueira was being trained to work with *T. cruzi* in Hertha Meyer's lab. Subsequently, she moved to Rockefeller University in New York, where she started a project in Zanvil Cohn's laboratory analyzing the interaction of *T. cruzi* with several different cell types, including mouse peritoneal macrophages, HeLa cells, L cells, and calf embryo fibroblasts. During a visit to Dr. Cohn's lab, one of the authors of the present paper (WS) had the opportunity to discuss these electron microscopy images with these investigators, which were subsequently published in a classic paper in 1976 (Nogueira and Cohn, 1976). The images showed clearly that all internalized parasites were initially located in a vacuole, designated the parasitophorous vacuole (PV) following the suggestion of William Trager, who described a vacuole akin to that in Nogueira and Cohn's images in erythrocytes infected by *Plasmodium* (Langreth and Trager, 1973). Similar observations were made in *T. gondii* by Jones and Hirsch (1972). Since that time, it has become clear that in all situations examined to date, *T. cruzi* and certain other intracellular protozoan always penetrate the host cell in a process that is better characterized as an endocytic process, involving the initial formation of aPV. This process takes place independent of the nature of the host cell. Even so-called non-professional phagocytic cells can be penetrated by *T. cruzi* and *T. gondii*. The term induced endocytosis or even induced phagocytosis has been used to refer to a process in which the parasite is able to stimulate endocytic activity in the future host cell.

According to Nogueira and Cohn in the case of *T. cruzi*, subsequent to penetration, the parasites leave the PV in a process they described as "escaping" and then enter into direct contact with the host cell cytoplasm. However, a few years later, it was shown (Carvalho and de Souza, 1989) that there is no escaping, but rather, fragmentation of the PV membrane occurs, most likely due to the activity of enzymes secreted by the parasite, as was subsequently shown by Andrews et al. (1990), associated with the complete disappearance of the PV. Taken together, these observations clearly indicate that an endocytic process is always involved during the process of *T. cruzi* internalization into a host cell. The same basic idea can be extended to other intracellular parasitic protozoa.

### A NEW CONCEPT OF "ACTIVE PENETRATION"

We had the opportunity to discuss the data described above as well as the results obtained by several groups in *Plasmodium* and *Toxoplasma* with Hertha Meyer, James Dvorak, Nadia Nogueira, and Zanvil Cohn, and we reached the conclusion that active penetration, as initially defined, does not exist during the process of parasitic protozoa interacting with host cells. A possible exception is observed during infection of host cells by microsporidians, which include a large number of species initially considered to be protozoans but that have more recently been considered to be fungi based on the presence of a large number of genes that, upon phylogenetic analysis, cluster Microsporidia with Fungi (see review by Xu and Weiss, 2005). These organisms present a complex life cycle, and their spores contain a structure known as the polar tube. When one of these organisms attaches to the surface of a host cell, signals activate a process that leads to evagination of a structure known as the polar tube, which then penetrates into the

host cell, establishing a type of bridge between the cytoplasm of the spore and the cytoplasm of the host cell. The sporoplasm, which may contain one or two nuclei, then flows through the connecting tube and is transferred to the host cell cytoplasm, where it will develop.

After some discussion, it was concluded that the term active penetration could be used to indicate the fact that the parasite plays an important role in the "induction of the host cell invasion process." Indeed, since the first description of the process, it has been clear that the intense movement of the protozoan, especially due to the flagellar beating process, plays some role. This idea was further analyzed by Schenkman et al. (1991), who showed that maintenance of an active energetic metabolism is fundamental for *T. cruzi* to invade cells, as this process is prevented by treatment of the parasites with 2-deoxy-glucose, an inhibitor of glycolysis, as well as sodium azide, antimycin, and oligomycin, which interfere with the mitochondrial metabolism involved in the synthesis of ATP.

The term active penetration has also been employed from another perspective. Kipnis et al. (1979), for example, used it to describe the penetration of bloodstream trypomastigotes into macrophages in a process that was only partially inhibited by cytochalasin B. At present, we know that this compound does not inhibit all forms of endocytosis.

Therefore, we can conclude that the available data, especially those obtained through transmission electron microscopy of thin sections, clearly show that recently penetrated *T. cruzi* of both infective and non-infective forms are always located within a PV that interacts with the organelles of the endosomal-lysosomal system during its short existence. This phenomenon occurs in all the cell types examined to date independent of whether they are professional or non-professional phagocytic cells. The formation of the PV involves the induction of a calcium flux into the host cell via the action of a parasite-derived calcium agonist, which is generated through the action of a parasitic oligopeptidase (Caler et al., 1998), as well as the synaptotagmin VII pathway (Caler et al., 2001), the recruitment of lysosomes (Tardieux et al., 1992), and the participation of microtubules (Tyler et al., 2005) and actin filaments (Rosestolato et al., 2002). Recently, Fernandes et al. (2011) showed that *T. cruzi* trypomastigotes mimic the process of wound repair with  $\text{Ca}^{2+}$ -dependent exocytosis of lysosomes by delivering acid sphingomyelinase to the host plasma membrane, facilitating parasite entry into host cells. These aspects of the invasion process have been extensively reviewed in recent years (Hall, 1993; Burleigh and Andrews, 1995; Yoshida, 2006; Alves and Colli, 2007; de Souza et al., 2010; Caradonna and Burleigh, 2011; Butler and Tyler, 2012; Fernandes and Andrews, 2012; Romano et al., 2012).

### ENDOCYTOSIS IS A COMPLEX BIOLOGICAL PROCESS

Our present knowledge of the endocytic process shows that it is more complex than previously thought. Indeed, in addition to the classical phagocytic process, there are several ways a cell can ingest extracellular material of variable dimensions. These mechanisms can be either dependent on dynamin, such as the clathrin- and caveolin-mediated processes, or independent of dynamin, as occurs during processes including macropinocytosis, and lipid

raft-mediated endocytosis. It is likely that other mechanisms will be described in view of the large number of groups attempting to better characterize the endocytic process. As described in another review in this volume (Barrias et al., submitted), *T. cruzi* may use all of these mechanisms to enter host cells. It is possible that the parasite selects the mechanism to be used based on factors such as the nature of the cell and the host cell surface ligand to which

binds. Once the parasite binds to and is recognized by the host cell surface it triggers a process that is better described as an induced endocytosis.

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